Cyclobutane-type Pyrimidine Photodimer Formation and Induction of Ornithine Decarboxylase in Human Skin Fibroblasts after UV Irradiation

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Cyclobutane-type pyrimidine photodimers as well as the induction of ornithine decarboxylase (ODC) may serve as biochemical markers of the mutagenic and carcinogenic effects of ultraviolet light (UV). For this reason, it is important to compare the formation of pyrimidine dimers with the induction of ODC in human skin fibroblasts after irradiation with UVC (200–290 nm) and UVB (290–320 nm). In our studies we determined cytosine-thymine (C-T) as well as thymine-thymine dimer yields (T-T) by high-pressure liquid chromatography in cultures of neonatal normal human foreskin-derived fibroblasts after irradiation with UVC and UVB light. It was found that the yield of dimerization and the ratio of T-T/C-T decreased from the UVC to the UVB region. Time-course studies of ODC-induction in the same cells indicated that the maximal activity after UVB irradiation was retarded compared to UVC exposure. For the UV-induced ODC-levels, however, no significant difference in maximal induction could be measured after UVC and UVB irradiation at fluences where comparable yields of thymine dimerization are produced. Similar ODC-maxima were obtained with strains from children, while cells from adults showed significantly less pronounced ODC induction, indicating that ODC-response decreases with age and may therefore be used as a marker of aging. *J Invest Dermatol 91:579–584, 1988*

It is well known that far-ultraviolet light (UVC) in the range of the absorption maximum of DNA electronically excites the heterocyclic basis and induces the formation of cyclobutane-type pyrimidine dimers. In the UVB region electronic excitation still occurs, but indirect mechanisms of DNA damage gain in relative importance [1]. Nevertheless, cyclobutane-type dimerization represents the predominant photoreaction even in the UVB. Ornithine decarboxylase (ODC) is involved in the synthesis of polyamines, which play a role in the regulation of DNA-synthesis and cell proliferation. Therefore, ODC may participate in the modulation of gene expression. Experimental evidence has shown that induction of ODC and formation of cyclobutane-type pyrimidine photodimers are implicated in UV-induced mutagenicity and carcinogenesis [2–5]. On this basis, ODC-induction and formation of pyrimidine dimers have been currently used for the evaluation of the protecting effects of sunscreens [6,7]. Recently, Sutherland and Griffin [8] reported photosensitization of pyrimidine dimer formation in bacterial and mammalian DNA by para-aminobenzoic acid (PABA), which is sometimes used in sunscreens. PABA is also known to influence the formation of free radicals, which might attack DNA by indirect action resulting in the formation of DNA-adducts [9]. Formation of indirect action involves the intermediacy of active oxygen species, which may also be formed by UVB light and influence the ODC-response [10]. We have previously observed differences in the thymine dimer distribution in chromatin following UVC and UVB irradiation [11]. Because it is evident that photochemical and photobiologic data obtained in the UVC cannot be directly extrapolated to the UVB, it is of interest to examine thymine dimer yields and correlate them with ODC-induction in human skin fibroblasts after irradiation with UVC and UVB light.

In this paper we report our results on the formation of UV-light induced cyclobutane-type pyrimidine photodimers in human skin fibroblasts using high-pressure liquid chromatography as described elsewhere [12]. Little work has been done regarding ornithine decarboxylase induction in human skin fibroblasts [13], and in the literature reports about the influence of UV-light on ODC in these cells are lacking. In order to be able to correlate pyrimidine dimer formation with UV-induced ODC-response, we determined how UVC and UVB may influence the time course of ODC-induction in human skin fibroblasts. Referring to this study, the maximal levels of induced ODC-activity were measured in several human skin fibroblast strains following exposure to different fluences of UVC and UVB light.

MATERIALS AND METHODS

Cells and Culture Conditions Skin fibroblasts from normal individuals (CRL 1221 and CRL 1222) and from a Xeroderma pigmentosum patient of complementation group A, XP12BE (CRL 1223), derived from a 10-year-old female, were obtained from the American Type Culture Collection. CRL 1222 were originally de-

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Abbreviations:
C-T: cyclobutane-type photodimer between cytosine and thymine
DMEM: Dulbecco's modified Eagle's medium
HPLC: high pressure liquid chromatography
MES: 2-[N-Morpholino]ehtanesulfonic acid
$NaBH_4$: sodium borohydride
Py-T: cyclobutane-type pyrimidine dimer containing thymine
T-T: cyclobutane-type thymine-thymine photodimer with cis-syn stereochemistry
U-T: cyclobutane-type photodimer between uracil and thymine
UV: ultraviolet light
UVB: UV-light from 290-320 nm
UVC: UV-light of wavelength shorter than 290 nm
XPA: xeroderma pigmentosum skin fibroblasts of complementation group A.

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rived from a 8-year-old-male and CRL 1221 from a 40-year-old male. The normal human skin fibroblast strains, GM 38 and GM 1717, were purchased from the Human Genetic Mutant Cell Repository (Camden, NJ) and originated from a 9-year-old black girl and a 39-year-old man, respectively. Neonatal foreskin-derived normal human fibroblasts 3229 were a gift from Dr. Robert Zimmerman who established this strain in the laboratory of Dr. John Little (Boston, MA) [14,15]. The passage numbers (1 : 2 split ratios) of the normal strains were 3229, passages (p) 31-47 and 110-122; CRL 1222, p 20-28; GM 38, p 21-53; GM 1717, p 16-28; CRL 1221, p 14-22. The passage numbers of the XP 12 BE strain CRL 1223 were 19-21. Fibroblasts to be irradiated were grown as monolayers in 6-cm-diameter Petri dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and 100 Uml^{-1} penicillin-streptomycin as described previously [12,16]. 24-36 h after seeding cells were labeled with ^{3}H-thymidine (2-10 μCi/ml, 91 Ci $mmol^{-1}$, Amersham, Zürich, Switzerland) overnight. The radioactive medium was then replaced by fresh medium and the cells incubated in a CO_2 incubator (Heraeus) until UV irradiation. For the determination of ODC 3×10^5 cells were seeded into Petri dishes and grown for 1 week until confluency. Fibroblasts were then held in the confluent state until UV-irradiation and subsequent performance of the ODC-assay described below.

Irradiation Facilities Medium was removed from the Petri dishes prior to irradiation, the cells were washed with Eagle's balanced salt solution (EBSS) and covered with 1.5 ml of the same solution. UVC light was provided by a 6 W germicidal (low-pressure Hg vapour) lamp from Philips, emitting the majority radiant energy at 254 nm wavelength. During individual experiments the fluence rate of 0.2 W m^{-2} was stable, as continually monitored by a IL 770 A germicidal-erythemal radiometer equipped with a SEE 400 photodetector containing a NBS 254 interference filter (International Light, Newburyport, MA). For UVB experiments an unfiltered UVB source from Osram (Ultra-Vitalux) was used. The Osram Ultra-Vitalux is an intermediate pressure, UVC filtered, mercury vapor lamp, which has a mercury vapor line spectrum and was extensively described by Sayre and Aging [17]. This lamp emits 0.5% and 2.6% of the total irradiance in the UVB and UVA range, respectively. The fluence rate was determined with the radiometer as already described. The SEE 400 photodetector, however, contained a NS 297 interference filter with half-power points between 290-301 nm. The measured fluence rate at 297 nm was 3 W m^{-2}. This value was used in our experiments. Using the UVB-probe from International Light frequently cited in the literature the fluence rate was 5.3 W m^{-2}, almost two-fold higher. The cells were irradiated on a rotating platform at ambient temperature.

DNA Isolation and Determination of Thymine-containing Pyrimidine Dimers by HPLC After irradiation the cells were harvested by trypsinization and DNA was isolated by the filter method described previously [18]. DNA was hydrolyzed with formic acid at 170°C for 90 min [19]. During this process cytosine-containing pyrimidine dimers were deaminated to yield uracil-containing pyrimidine dimers [20]. Thymine-thymine (T-T) and uracil-thymine dimers (U-T) were separated and isolated from formic acid hydrolysates on two μBondapak C_{18} columns in series (Waters Associates, Zurich, Switzerland) as described previously [11,12]. The isolated dimers were reduced according to our previous published procedure [12,21]. Separation of the reduced reaction mixture was carried out on the HPLC radial compression system (RCM 100 module, Waters) containing a C_{18} resolve cartridge (packed with 10 μ particles). Elution was with 2% methanol under isocratic conditions at a flow rate of 1 ml/min^{-1}. Fractions of 0.5 ml were collected in 7-ml mini-vials (Packard, Zürich, Switzerland) and supplemented with 5 ml Optifluor (Packard). Then the radioactivity content of the 80 fractions was determined in the Tricarb 2050 scintillation system from Packard.

Ornithine Decarboxylase Assay ODC activity in cell lysates was determined essentially as described elsewhere [22,23]. In short, 4-48 h following irradiation the confluent monolayers were washed with assay buffer (2mM EDTA, 5 mM DTT, 400 μM pyridoxal phosphate in 50 mM MES (2-[N-Morpholino]ethanesulfonic acid) pH 6.9) and scraped off with a rubber policeman. For each experimental point the contents of two to five 6-cm diameter Petri dishes were pooled. The cells were centrifuged (2000 rpm, 5 min) and the pellet was dissolved in 500 μl assay buffer as described above. The cells were stored in liquid nitrogen until ODC determination. On the day of analysis, cell suspensions were lysed by three cycles of freezing and thawing. The lysates were centrifuged at 12.000 × g for 30 min at 4°C, and the supernatants, which usually contained $\approx$ 1mg ml^{-1} protein, were assayed for their capacity to liberate CO_2 from D-L [1-^{14}C] ornithine (61 mCi/mmol, Amersham, Zürich, Switzerland). For this purpose, 100 μl of supernatant was put into each 15 ml tube followed by 100 μl of assay buffer and 100 μl of ornithine (2mM; 0.25μCi). The tube was then closed with a rubber stopper equipped with a center well (Kontes, Vineland, NJ) containing a glass microfiber filter (Whatman, GF/A, diameter 2.1 cm) and 200 μl CO_2 absorber [ethanolamine: methoxyethanol (v:v/2:1]. Reactions were performed for 1 h at 37°C with shaking. The reaction was then stopped by addition of 100 μl 5% sulfuric acid through the rubber stopper and shaking was continued overnight. The filters were placed in scintillation vials, covered with 0.8 ml ethanol, and 30 min later 10 ml Optifluor was added. Finally the filters were counted in the Tricarb 2050 scintillation system for 5 min.

RESULTS

Formation of Thymine-containing Pyrimidine Dimers in Human Fibroblasts In a previous report we determined cyclobutane-type pyrimidine photodimer formation and excision in human skin fibroblasts after irradiation with 313 nm monochromatic light [12]. We have extended this basic experiment measuring pyrimidine photodimer formation after UVC irradiation with a germicidal

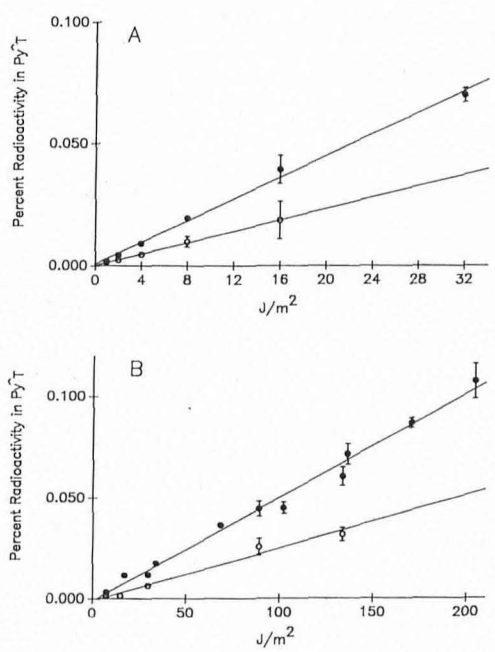

Figure 1. Formation of cytosine-thymine (C-T, *open circle)* and thymine-thymine (T-T, *closed circle)* dimers in DNA of foreskin-derived human skin fibroblasts upon irradiation with UV-light. Note that the values for C-T dimers represent the measured amount of radioactivity found in this region. In order to calculate the corrected percentage of C-T dimers these data have to be multiplied by a factor of 2, accounting for the fact that only the thymine of a C-T dimer could be labeled. Mean values of at least two analyses with standard deviation are given. *A:* Irradiation with a germicidal lamp emitting mainly 254 nm light. *B:* Irradiation with a UVB source (Osram; Ultra-Vitalux) emitting UVB near 290 nm.

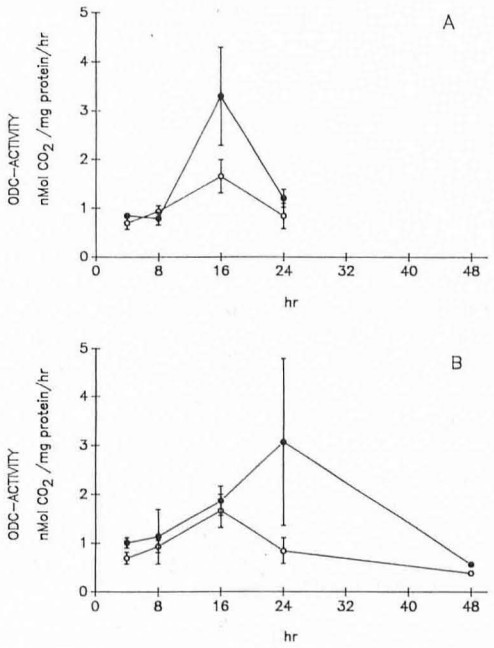

Figure 2. Time course of ODC-induction in foreskin-derived human skin fibroblasts by medium change alone *(open circle)* and after irradiation with UV-light and medium change *(closed circle)*. *A:* Irradiation with UVC light. Mean values of at least four analyses in two independent experiments with standard deviation are given. *B:* Irradiation with UVB light. Mean values of three independent experiments with standard deviation are given.

lamp and UVB irradiation using a UVB source (Ultra-Vitalux) of a type occasionally found in solariums. Our results are shown in Fig 1. Fluence response curves for C-T and T-T formation in monolayers of foreskin derived human skin fibroblast 3229 following UVC irradiation (part *A*) as well as after UVB irradiation (part *B*) are given in this Fig 1. Similar fluence response curves were obtained with the repair deficient XPA cells CRL 1223 [24]. From the slopes of the curves a rate of formation per Jm^{-2} for C-T and T-T of 0.0020% and 0.0023%, respectively, after UVC irradiation, was determined. After UVB irradiation 0.0005% per Jm^{-2} were found for C-T as well as T-T. The T-T/C-T ratios are UV-source dependent being 1.2 after UVC irradiation and 1.0 following UVB exposure, confirming our previously published data [24].

Ornithine Decarboxylase Induction in Human Fibroblasts

DiPasquale and co-workers [25] found a six-fold ODC-increase in human skin fibroblasts after treatment of cultures with epidermal growth factor (EGF). In our experiment, we found in foreskin derived human skin fibroblasts 3229 a fivefold increase after treatment with 1 ng/ml EGF (data not shown). Figure 2 represents a time course of ODC-induction after UVC and UVB irradiation in the same fibroblasts 3229 with fluences where similar dimer levels are produced. In parts *A* and *B* of this Fig 2 ODC induction in human cells after irradiation with 24 Jm^{-2} UVC and 90 Jm^{-2} UVB, respectively, are given. It has to be noted that the ODC induction peaked following irradiation with UVC after 16 h, while after UVB irradiation for ODC-induction the maximal activity was reached at 24 h. Similar results were obtained with the normal strains CRL 1222 and 1221 (data not shown). For the repair-deficient XPA cells CRL 1223 no discernible difference in ODC production was detected 4-24 h after medium changes or after 90 Jm^{-2} UVB irradiation as depicted in Fig 3.

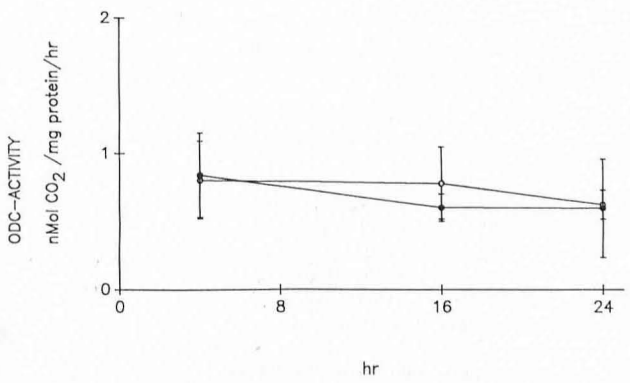

Figure 3. Time course of ODC-induction in repair deficient XPA cells CRL 1223 by medium change alone *(open circle)* and after UVB irradiation and medium change *(closed circle)*. Mean values of at least four analyses in two independent experiments with standard deviation are given.

Table I. ODC-induction and Pyrimidine Dimer Efficiency in Human Foreskin-derived Fibroblasts (3229) after UVC Irradiation

Fluence Jm^{-2}	ODC-Activity[a] %	Py-T yield[b] %
Control	100.0 ± 7.5 (n = 6)	—
6	97.4 ± 11.3 (n = 4)	0.026
12	114.3 ± 16.0 (n = 4)	0.052
24	213.1 ± 84.7 (n = 8)	0.103
30	225.4 ± 12.7 (n = 1)	0.129
36	326.1 ± 123.2 (n = 4)	0.155
48	98.0 ± 5.7 (n = 2)	0.206

[a] ODC activity in % of confluent human skin fibroblasts 16 h after medium change (control) or irradiation with UVC light. n means number of independent experiments in duplicate determinations.

[b] Thymine containing pyrimidine dimer densities are calculated from results represented in Fig 1 A.

Tables I and II compare ODC induction with pyrimidine dimer yields following irradiation with UVC and UVB light, respectively. It is evident that both pyrimidine dimer yields as well as enzyme activity increase in a fluence dependent manner in the UVC and UVB range of 0-36 and 0-135 Jm^{-2}, respectively. The significant decrease of ODC-response in skin fibroblasts with higher fluences is probably due to toxic effects of UV light. In order to evaluate the correlation of ODC response and yield of dimerization between UVC and UVB fluences, equidimer values of Table I were individually compared with those of Table II using the students' t-test for paired data. No significant difference for $p = 0.05$ was found, which supports the notion that there exists a good correlation between pyrimidine dimer formation and ODC response up to a level of 0.16% UV-induced pyrimidine dimers. Table III compares the UV-induced ODC response in human skin fibroblasts derived from young and adult donors. It is noteworthy that similar ODC-induction was obtained in the neonatal foreskin-derived human skin fibroblasts 3229 and the normal strains CRL 1222 and GM 38 from an 8- and 9-year-old donor, respectively. In the normal

Table II. ODC-induction and Pyrimidine Dimer Efficiency in Human Foreskin-derived Fibroblasts (3229) after UVB Irradiation

Fluence Jm^{-2}	ODC-Activity[a] %	Py-T yield[b] %
Control	100.0 ± 12.5 (n = 11)	—
45	106.0 ± 19.1 (n = 5)	0.046
90	262.6 ± 199.8 (n = 13)	0.090
113	306.2 ± 66.3 (n = 2)	0.114
135	227.6 ± 67.3 (n = 10)	0.136
180	87.2 ± 4.9 (n = 2)	0.180

[a] ODC activity in % in confluent human skin fibroblasts 24 h after medium change (control) or irradiation with UVB light. n means number of independent experiments in duplicate determinations.

[b] Thymine containing pyrimidine dimer densities are calculated from results represented in Fig 1 B.

Table III. Comparison of UV-induced ODC Response in Cells from Young and Adult Donors

Fluence Jm^{-2}	ODC-Activity in Young Donor Cells[a] %	ODC-Activity in Adult Donor Cells[b] %	Significance[c]
control	100.0 ± 12.0 (n = 7)	100.0 ± 6.6 (n = 6)	none
24 (UVC)	251.2 ± 83.3 (n = 6)	156.5 ± 29.0 (n = 5)	p = 0.05
control	100.0 ± 9.0 (n = 9)	100.0 ± 13.0 (n = 8)	none
45 (UVB)	104.7 ± 24.7 (n = 5)	96.4 ± 9.6 (n = 2)	none
90 (UVB)	284.5 ± 83.6 (n = 9)	124.5 ± 22.4 (n = 8)	p = 0.001
135(UVB)	535.6 ± 357.0 (n = 9)	153.9 ± 42.1 (n = 5)	p = 0.02

[a] Mean values of at least three and four independent experiments with cell strains GM 38 and CRL 1222, respectively, with standard deviation are given. n means number of total independent experiments in duplicate determinations.
[b] Mean values of at least two and three independent experiments with cell strains GM 1717 and CRL 1221, respectively, with standard deviation are given. n means number of total independent experiments in duplicate determinations.
[c] Student-t test for paired data used for calculation of significance.

cells GM 1717 and CRL 1221 derived from a 39- and 40-year-old donor, respectively, the ODC-stimulation was significantly less pronounced after both UVC and UVB irradiation. The repair-deficient XPA cells CRL 1223 showed no significant UV-induced ODC response (data not shown).

DISCUSSION

Various laboratories have reported UV-induced pyrimidine dimer yields measured either by chromatographic procedures or by assays measuring pyrimidine-dimer-specific endonuclease-sensitive sites. Table IV gives an overview for pyrimidine dimer yields obtained by chromatographic procedures upon irradiation with 254-nm light. It has to be noted that all the values presented in Table IV are calculated from the published data on the assumption that half of the total Py-T is T-T. Our values support the values of Williams and Cleaver (1978) [26], Zelle et al (1980) [31], and our previous published data [11,24,34]. They are somewhat lower than those of most other reports. The differences may be due to factors like irradiation sources and irradiation conditions. Nevertheless, we can conclude from this synopsis of the efficiency of pyrimidine dimerization that the germicidal UV-source used emits predominately 254-nm light.

The relevance and usefulness of different light sources to UVB photobiology depend on the question being asked. Therefore we used a UVB source from Osram (Ultra-Vitalux) because we estimate results closely related to the practical situation of solar exposure. This choice is further supported by studies of Sayre and Aging [17] who compared human sun protection factors to predicted protection factors using two differently filtered xenon arc solar simulators and the Osram Vitalux lamp system. Besides the light source, dimer yields in the UVB region are influenced by the irradiation temperature [21], and the nucleosomal linker DNA is enriched in dimer content by a factor of 2-4 relative to bulk DNA [11]. In contrast, neither temperature nor nucleosomal structure affect dimerization in the UVC region [34,11]. Our observations of high photodimer yield after UVB irradiation in this report confirm previous data from Suzuki et al [5], because unfiltered UVB sources emit relatively large amounts of short UVB wavelengths in the range of 290 nm. Although the UVB region in noon summer sunlight may represent only about 40% of the total cytotoxic effectiveness of sunlight at 290-434 nm [36] this region efficiently induces cyclobutane-type dimerization [24].

The time course of ODC-stimulation in human skin fibroblasts following UVC and UVB exposure showed a small but significant time-shift in response. As already mentioned it is difficult to extrapolate photochemical and photobiologic data obtained in the UVC to the UVB. In the UVB region photosensitization gains in importance relative to UVC, inducing complex chain reactions in which singlet oxygen and superoxide radicals participate [37]. In particular, interactions between UV and aromatic amino acids, riboflavins, and other biologic photosensitizers may influence the time course of ODC-induction. When the effect of photosensitizers is taken into account it does not seem surprising that the maximum of ODC-increase was retarded after UVB irradiation relative to UVC.

It is interesting that there is no significant difference for ODC induction by UVC and UVB light at fluences that produce similar pyrimidine dimer levels. This finding supports inquiries on UV-damage to skin in attempts to measure the photoprotective efficacy of topically applied sunscreens using ODC or pyrimidine dimers as marker [6,7]. As already mentioned little work has been performed regarding ODC induction in human skin fibroblasts and no data concerning UV-induced ODC response of human fibroblasts are reported. Several laboratories, however, have shown an UV-wave length dependence for the induction of ODC-activity in hairless mouse epidermis [38,39]. From these data no clear picture has evolved about the chromophore for ODC-induction. While Young et al [38] assumed a protein as a chromophore, the results of Kligman and Kaidby [39] tend to identify DNA as the chromophore. Our results of a correlation between ODC-response and

Table IV. Compilation of Thymine Dimer Yields after Irradiation of Mammalian Cells with 254-nm Light Using Chromatographic Procedures

Organism	Assay	% T-T per Jm^{-2}	References
CV-1	TLC	0.0020	Williams and Cleaver, 1978 [26]
V-79	TLC	0.0029	Ahmed and Setlow, 1979 [27]
GM 38	TLC	0.0035	Ehmann et al, 1978 [28]
V-79	PC	0.0028	Rothmann and Setlow, 1979 [29]
HSBP	PC	0.0034	Regan et al, 1978 [30]
CV-1	PC	0.0023	Zelle et al, 1980 [31]
XP 12BE	TLC	0.0031	Kantor et al, 1980 [32]
WI 38	PC	0.0036	Regan et al, 1968 [33]
CRL 1221	HPLC	0.0024	Niggli and Cerutti, 1982 [11]
CRL 1223	HPLC	0.0022	Niggli, 1986 [34]
CRL 1223	HPLC	0.0024	Niggli et al, 1988 [24]
GM 38	HPLC	0.0033	Weinfeld et al, 1986 [35]

pyrimidine dimer formation at least for dimer levels up to 0.16% support the notion that DNA is the primary chromophore involved. This conclusion is further supported by the data of Lowe et al [40] and Ley [41], who found a correlation between UV-induced erythema and ODC response or pyrimidine dimer formation, respectively.

The observation that ODC in fibroblasts from younger donors can be significantly more stimulated compared to older donors supports the data from Chen et al [42]. They found less ODC-activity response in human fibroblasts with increasing passage number. In this respect it is fascinating that the repair deficient XPA cells lacked UV-stimulated ODC induction. XP patients are said to manifest premature aging of the skin, although published descriptions are lacking (for review see Ref 43). Our data may point to a relationship between aging and this autosomal recessive disorder. A possible explanation for this connection may be, as shown by many sources, that the control of gene expression in higher organisms is related to the methylation of cytosine in DNA, and that the pattern of methylation is inherited [44]. In this respect Steglich et al [45] provided data that ODC in some hamster cell lines is inactivated by DNA methylation. Diminution of ODC-response in normal aging cells from adult donors and loss of ODC induction in XP-fibroblasts is therefore the result of DNA methylation. These observations and interpretations are also consistent with the stem-cell system differentiation theory proposed by Bayreuther et al [46,47], who demonstrated that moderate fluences of UV-light accelerate the differentiation pathway from mitotic to postmitotic fibroblasts [48-50]. In conclusion, our studies showed that UV-light is important for the induction of ODC activity in human skin fibroblasts. The correlation with pyrimidine dimer formation suggests DNA rather than protein as a chromophore. The role of ODC induction in phototumorigenesis, however, remains unknown and requires further investigations.

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NIH WORKSHOP REPORT ON THE NON-DERMATOLOGICAL COMPLICATIONS OF EPIDERMOLYSIS BULLOSA

In September 1986 the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), National Institutes of Health, funded a workshop on the Non-Dermatological Complications of Epidermolysis Bullosa (EB). After hearing presentations by a panel of scientific experts on such subjects as basic skin biology, wound healing, esophageal strictures and webbing, complications related to malnutrition, gastrointestinal manifestations, eye lesions, dental involvement, and orthopedic deformities, a comprehensive report was prepared and published. Free, single copies of this report are available from: Arlene Pessar, R.N., D.E.B.R.A. of America, Inc., 451 Clarkson Avenue—Room E6101, Brooklyn, New York 11203. Telephone: (718) 774-8700